Journal of the American College of Cardiology Vol. 48, No. 1, 2006

Published by Elsevier Inc.
ISSN 0735-1097/06/$32.00
doi:10.1016/j.jacc.2006.03.035

Atorvastatin Increases Plasma Soluble Fms-Like Tyrosine Kinase-1 and Decreases Vascular Endothelial Growth Factor and Placental Growth Factor in Association With Improvement of Ventricular Function in Acute Myocardial Infarction

Yasushi Kodama, MD, Yoshinobu Kitta, MD, Takamitsu Nakamura, MD, Hajime Takano, MD, PHD, Ken Umetani, MD, PHD, Daisuke Fujioka, MD, Yukio Saito, MD, Ken-ichi Kawabata, MD, Jyun-ei Obata, MD, PHD, Akira Mende, MD, Tsuyoshi Kobayashi, MD, Kiyotaka Kugiyama, MD, PHD

Yamanashi, Japan

OBJECTIVES This study examined whether atorvastatin increases plasma levels of soluble Fms-like tyrosine kinase 1 (sFlt-1) and reciprocally decreases vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) levels in patients with acute myocardial infarction (AMI).

BACKGROUND Statins exert cardioprotective actions partly through anti-inflammatory actions. By capturing VEGF and PlGF in plasma, sFlt-1 acts as a natural inhibitor of VEGF and PlGF, which have proinflammatory properties.

METHODS Left ventriculography and enzyme-linked immunosorbent assay of plasma levels of sFlt-1, VEGF, and PlGF were repeated after AMI in 50 consecutive patients with a first AMI. Patients were randomized to treatment with atorvastatin (10 mg/day; n = 25) or placebo (n = 25) within 3 days after AMI, and therapy was continued for 6 months.

RESULTS The sFlt-1 levels were low in the acute phase, followed by an increase at 2 weeks after AMI, whereas free VEGF and PlGF levels were high in the acute phase, followed by a decrease at 2 weeks. Atorvastatin increased sFlt-1 levels and reciprocally decreased VEGF and PlGF levels at 6 months compared with placebo. The increase in sFlt-1 levels and the decrease in VEGF and PlGF levels were correlated with improvement of left ventricular ejection fraction during the follow-up period.

CONCLUSIONS There was a reciprocal relationship between changes in sFlt-1 levels and changes in VEGF and PlGF levels after AMI; and atorvastatin increased sFlt-1 levels while decreasing VEGF and PlGF levels. These changes were associated with late improvement of post-MI ventricular function, and may represent an additional benefit of statin therapy. (J Am Coll Cardiol 2006;48:43–50) © 2006 by the American College of Cardiology Foundation

The Fms-like tyrosine kinase 1 (Flt-1), a receptor for vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), can also be produced as a soluble protein (sFlt-1) which lacks both the membrane-spanning and intracellular tyrosine kinase domains of Flt-1 (1–4). Soluble Flt-1 is generated by alternative splicing of the Flt-1 gene in vascular endothelium as well as placenta (4,5). Soluble Flt-1 captures VEGF and PlGF in plasma and reduces the amount of free VEGF and PlGF available to bind to their vascular endothelial receptors (4–7). Therefore, sFlt-1 levels could potentially determine the free plasma levels of VEGF and PlGF and their pathophysiologic activities (4–11).

It has been shown that the exogenous administration of VEGF and PlGF was effective in causing therapeutic angiogenesis in animal models and in patients with coronary artery disease (CAD) (12,13). However, Flt-1, a receptor for VEGF and PlGF, is expressed on monocytes/macrophages as well as vascular endothelium (1–3). Thus, VEGF and PlGF also have proinflammatory properties and promote monocyte activation and migration through Flt-1 (1–3). In this context, VEGF and PlGF have proatherogenic activity as well as antiatherogenic activity; therefore, the role of the endogenous VEGF and PlGF in the long-term clinical outcome of CAD remains unclear. In fact, elevated levels of endogenous VEGF or PlGF were associated with an adverse prognosis in patients with acute coronary syndrome (ACS) (14,15). Therefore, levels of sFlt-1, a natural antagonist of VEGF and PlGF, may also provide important prognostic information in ACS. A previous report (9) showed that plasma sFlt-1 levels were decreased within 24 h after thrombolytic therapy in patients with acute myocardial infarction (AMI), but sFlt-1 levels were not measured over several months and there was no assessment of the clinical significance of the sFlt-1 changes.

Statins are shown to exert beneficial effects on the clinical outcome of ACS partly through anti-inflammatory actions in addition to their lipid-lowering effects (16,17). A previous study (18) showed that atorvastatin decreased the plasma levels of free VEGF in patients with CAD in the absence of AMI, but the study did not examine sFlt-1 levels and the possible

From the Department of Internal Medicine II, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan. Supported by grants-in-aid for (B)(2)-15390244, Priority Areas (C) Medical Genome Science 15012222 from the Ministry of Education, Culture, Sports, Science, and Technology, Health, and Labor Sciences Research Grants for Comprehensive Research on Aging and Health (H15-Choju-012), Tokyo, Japan.

Manuscript received January 6, 2006; revised manuscript received February 22, 2006, accepted March 6, 2006.

Abbreviations and Acronyms

ACS = acute coronary syndrome
AMI = acute myocardial infarction
CAD = coronary artery disease
Flt-1 = Fms-like tyrosine kinase 1
LDL = low-density lipoprotein
LVEDVI = left ventricular end-diastolic volume index
LVEF = left ventricular ejection fraction
LVESVI = left ventricular end-systolic volume index
MI = myocardial infarction
PlGF = placental growth factor
VEGF = vascular endothelial growth factor

clinical significance of the decrease in VEGF levels. Therefore, we hypothesized that statin treatment may affect serial changes in the plasma levels of sFlt-1, VEGF, and PlGF over the time course of AMI, leading to an improvement of the depressed cardiac function that occurs after AMI. In this study, we determined the effect of atorvastatin on plasma levels of sFlt-1, VEGF, and PlGF over 6 months in patients with AMI and examined the relation between changes in the plasma levels of these factors with post-MI left ventricular function.

METHODS

Study patients. This study included 50 consecutive patients with a first AMI who were admitted within 24 h after the onset of symptoms and had successful reperfusion therapy with percutaneous coronary intervention immediately after admission to Yamanashi University Hospital. The diagnosis of AMI was made on the basis of chest pain persisting for ≥30 min, ST-segment elevation of >0.2 mV in ≥2 contiguous leads on a standard 12-lead electrocardiogram, and elevation of serum creatine kinase levels to more than twice the upper limit of normal. The study excluded patients with total cholesterol levels of >240 mg/dl or use of lipid-lowering medications at admission. The study also included 20 control subjects selected from the consecutive 27 subjects with angiographically normal coronary arteries and a normal left ventriculogram who underwent cardiac catheterization at Yamanashi University Hospital during the same study periods as patients with AMI. They were selected to match the AMI patients for atherosclerosis risk factors, and plasma levels of sFlt-1, VEGF, and PlGF were compared between the AMI patients and control subjects. The clinical characteristics of the patients with AMI and the control subjects are shown in Table 1. Written informed consent was obtained from all patients and control subjects before the study. The study was in agreement with the guidelines approved by the ethics committee at our institution.

Study protocol and blood sampling. The patients with AMI were randomly assigned to receive 6 months of oral atorvastatin (10 mg/day) or placebo (similar-appearing tablet) using a random number table generated by a computer.

Table 1. Patients' Characteristics at Baseline

	Acute Myocardial Infarction			
	Atorvastatin (n = 25)	Placebo (n = 25)	Controls (n = 20)	p Value
Age (yrs)	64.5 ± 2.1	62.7 ± 2.2	64.1 ± 1.1	NS
Male (%)	68	72	75	NS
BMI (kg/m^2)	23.2 ± 0.6	24.5 ± 0.6	24.5 ± 0.6	NS
Smoking (%)	56	48	55	NS
Hypertension (%)	72	64	70	NS
Diabetes mellitus (%)	60	64	55	NS
Total cholesterol (mg/dl)	196 ± 8	202 ± 8	198 ± 5	NS
Triglyceride (mg/dl)	133 ± 17	143 ± 12	124 ± 8	NS
HDL cholesterol (mg/dl)	46 ± 3	45 ± 3	49 ± 2	NS
LDL cholesterol (mg/dl)	120 ± 6	116 ± 7	115 ± 4	NS
Location of MI (%)				
Anterior	52	56	—	NS
Inferior	28	20	—	NS
Others	20	24	—	NS
Extent of CAD (%)				
1-vessel	36	48	—	NS
2-vessel	40	32	—	NS
3-vessel	24	20	—	NS
Killip classification (%)				
Class I	72	64	—	NS
Class II to IV	28	36	—	NS
Peak CK-MB (ng/ml)	266 ± 45	294 ± 77	—	NS
Time to reperfusion (h)	6.4 ± 0.9	6.1 ± 0.8	—	NS

Values represent the percent of the patients and control subjects or mean ± SE. Smoking defined as smoking ≥10 cigarettes/day for ≥10 years; hypertension defined as >140/90 mm Hg or use of antihypertensive medication; diabetes mellitus defined according to the American Diabetes Association report or as taking an antidiabetic medication.

BMI = body mass index; CAD = coronary artery disease; CK = creatine kinase; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MI = myocardial infarction.

All patients were blinded to the content of the tablets. The treatment was started within 3 days after admission. All patients received instruction and counseling to promote compliance with lipid-lowering diet according to the National Cholesterol Education Program Adult Treatment Panel III guideline. Patients were not permitted to be treated with any lipid-modifying drugs other than the study drug.

At the acute phase of MI, blood samples were obtained from an antecubital vein without stasis in all patients immediately after admission and before the administration of heparin. In 32 patients with AMI admitted within 6 h after symptom onset, blood samples were taken every 4 h over the first 24 h for determination of creatine kinase levels. Furthermore, blood samples were taken at 2 weeks and 6 months after AMI in the same manner from the antecubital vein without stasis in all patients with AMI. In the control subjects, blood samples were obtained from the antecubital vein without stasis. The blood samples, anticoagulated with EDTA, were immediately centrifuged at 3,000 rpm for 10 min at 4°C, and an aliquot of the EDTA-plasma was stored at −80°C until analyzed. Serum from a peripheral vein was also obtained at the same time.

Assays. Plasma levels of sFlt-1 were measured by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, Minnesota) according to the manufacturer's instruction. The sFlt-1 ELISA system is capable of detecting the total amount of plasma sFlt-1, including that bound to VEGF and PlGF. Plasma levels of free VEGF and PlGF (not bound to sFlt-1) were measured by sandwich ELISAs (R&D Systems) (5,11,19). Both free and sFlt-1–bound VEGF levels (total VEGF levels) were measured by competitive immunoassay (Accucyte; Cytimmune Science, Rockville, Maryland) (19,20). The minimal detection limits for sFlt-1, free VEGF, free PlGF, and total VEGF levels were 14.4 pg/ml, 9 pg/ml, 7 pg/ml, and 0.195 ng/ml, respectively. The C-reactive protein levels in the serum were assayed by rate nephelometry (Dade Behring, Marburg, Germany). Plasma levels of interleukin-8 were measured by ELISA (Quantikine; R&D Systems). These assays were performed by an investigator blinded to the sources of the samples.

Cardiac catheterization. Cardiac catheterization was performed immediately after admission at the acute phase of AMI and at 2 weeks and 6 months after AMI in all patients with AMI. Left ventriculography was performed at 2 weeks and 6 months after AMI. Left ventricular ejection fraction (LVEF), left ventricular end-diastolic volume index (LVEDVI), and left ventricular end-systolic volume index (LVESVI) were determined with left ventriculograms by area-length methods using computer-assisted analysis (Cardio 2000; Fukuda-denshi Corp., Tokyo, Japan).

Statistical analysis. Data are expressed as mean ± SEM unless otherwise indicated. The mean value and frequency between two groups were compared using Student unpaired *t* test and chi-squared test, respectively. When frequencies among the patients with atorvastatin treatment, patients with placebo, and control subjects were compared (Table 1), chi-squared test using a 2×3 contingency table was initially examined for independence of frequencies among the three groups. When significant in independence by chi-square test, wholly significant difference was calculated (Tukey test) between the two groups. One-way analysis of variance followed by a Scheffe test for post hoc comparisons was used for comparisons of mean values before and during the treatment and of mean values among the three patient groups. The mean values of lipid and other biochemical parameters and of cardiac function before and during treatment in the two AMI groups were compared using two-way analysis of variance for repeated measures followed by post-hoc testing with a Scheffe test. The relationships of sFlt-1, VEGF, and PlGF levels, ventricular function, and other parameters was examined by linear regression analysis. Statistical significance was defined as $p < 0.05$. Analyses were performed in part using StatView 5.0 for Windows (Tokyo, Japan).

RESULTS

Comparisons of baseline clinical characteristics. The study patients with AMI were randomly assigned to atorvastatin treatment in 25 patients and placebo in 25 patients. All of the study patients completed the trial. The clinical characteristics of the patients with AMI and the control subjects are shown in Table 1. Risk factor profiles were similar among the two treatment groups and the control subjects, as shown in Table 1. Clinical parameters associated with AMI were comparable between the two treatment groups with AMI, as shown in Table 1.

In the acute phase of MI (within 24 h after AMI), sFlt-1 plasma levels were lower in patients with AMI than in control subjects, whereas free VEGF and PlGF levels were higher, as shown in Figures 1A to 1C. The placebo- and atorvastatin-treated groups with AMI had comparable levels of sFlt-1, free VEGF, and free PlGF in the acute phase of MI, as shown in Figures 1A to 1C.

Effects of treatments with atorvastatin and placebo during the follow-up period in patients with AMI. The effects of the treatments on lipid profiles, cardiac function, and other clinical features are shown in Table 2. The levels of total cholesterol and low-density lipoprotein (LDL) cholesterol were significantly lower in the atorvastatin group than in the placebo group at 6 months after AMI, but the levels were comparable between the two treatment groups at 2 weeks after AMI. The frequencies of each of the cardiac medications were similar except for atorvastatin between the two treatment groups during the follow-up period.

Plasma levels of sFlt-1, free VEGF, and free PlGF were not significantly different among the two treatment groups and the control subjects at 2 weeks after AMI, as shown in

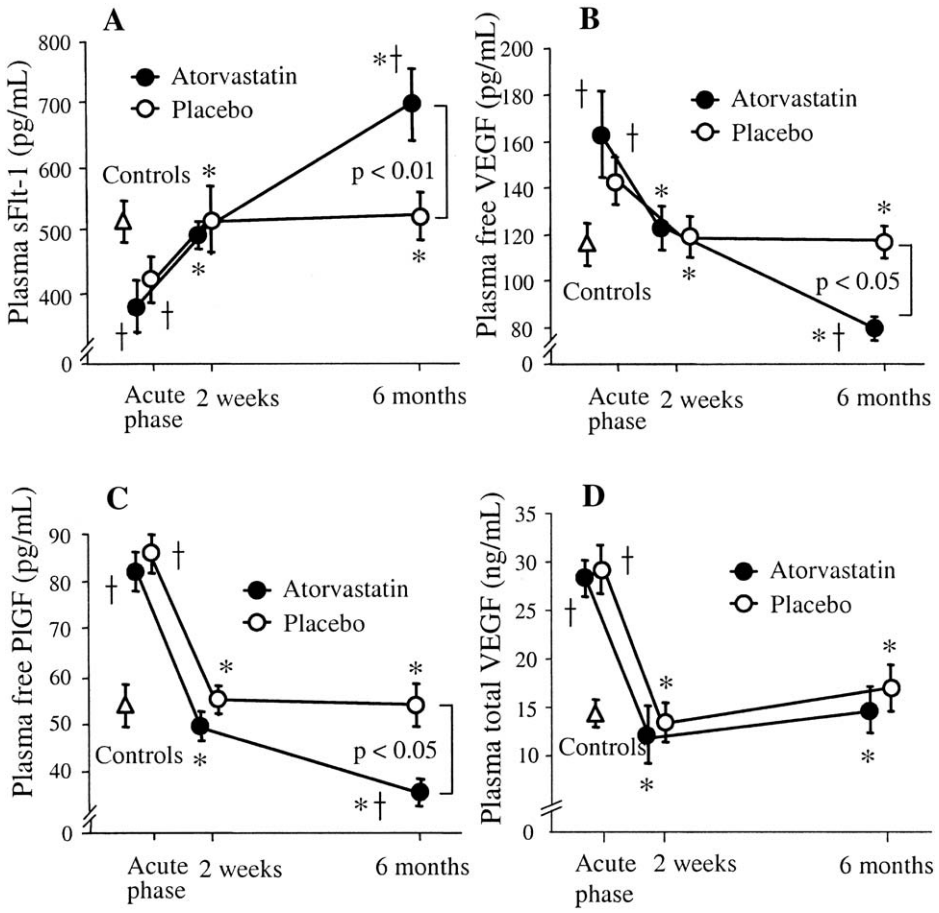

Figure 1. Comparison of soluble Fms-like tyrosine kinase-1 (sFlt-1) **(A)**, free vascular endothelial growth factor (VEGF) **(B)**, free placental growth factor (PlGF) **(C)**, and total VEGF **(D)** levels during the follow-up period after AMI in patients treated with atorvastatin (n = 25) and placebo (n = 25). *$p < 0.05$ vs. respective levels in the acute phase of myocardial infarction. †$p < 0.05$ vs. healthy control subjects.

Figures 1A to 1C. The plasma levels of sFlt-1, free VEGF, and free PlGF were unchanged from 2 weeks to 6 months after AMI in the placebo group. However, in the atorvastatin group, sFlt-1 levels increased from 2 weeks to 6 months after AMI, whereas free VEGF and free PlGF levels reciprocally decreased from 2 weeks to 6 months after AMI, as shown in Figures 1A to 1C. Total VEGF levels had similar changes during the study period as free VEGF levels (Fig. 1D). However, there was no significant difference in total VEGF levels between the two treatment groups (Fig. 1D). As shown in Figure 2, the relative changes in the plasma levels at 2 weeks and 6 months after AMI to the levels at acute phase of MI (=100) between the two treatment groups remained similar between the two treatment groups to the absolute changes shown in the Figure 1.

The LVEF in the atorvastatin group was similar at 2 weeks after AMI but significantly greater at 6 months after AMI compared with the placebo group, as shown in Figure 3 and Table 2. The LVEDVI and LVESVI in the atorvastatin group showed a tendency to be lower at 6 months after AMI than in the placebo group, but not reaching significance, as shown in Table 2.

Correlation of changes in levels of sFlt-1, VEGF, or PlGF and ventricular function during the treatment in patients with AMI. The percentage changes in LVEF were correlated positively with the percentage changes in sFlt-1 levels and inversely with the percentage changes in free VEGF and free PlGF levels from 2 weeks to 6 months after AMI, as shown in Figure 4.

The percentage changes in interleukin-8 levels were significantly correlated positively with percentage changes in free VEGF and free PlGF levels and inversely with sFlt-1 levels from 2 weeks to 6 months ($r = 0.31$, 0.35, and −0.29, respectively; all $p < 0.05$). The percentage changes in LDL cholesterol and C-reactive protein levels did not show a significant correlation with percentage changes in sFlt-1, free VEGF, and free PlGF levels and LVEF from 2 weeks to 6 months after AMI (LDL cholesterol levels: $r = -0.1$, 0.1, 0.05, and −0.14, respectively; C-reactive protein levels: $r = 0.15$, −0.19, 0.2, and 0.05, respectively).

DISCUSSION

The present study showed that sFlt-1 levels were elevated and free VEGF and free PlGF levels were reciprocally

Table 2. Risk Factors, Cardiac Functions, and Medications During the Follow-Up Period in Patients With Acute Myocardial Infarction

	Atorvastatin (n = 25)		Placebo (n = 25)	
	2 Weeks	6 Months	2 Weeks	6 Months
Risk factors				
Total cholesterol (mg/ml)	201 ± 8	166 ± 8*†	209 ± 8	201 ± 8
LDL cholesterol (mg/ml)	130 ± 6	87 ± 8*†	122 ± 7	116 ± 7
HDL cholesterol (mg/ml)	49 ± 3	54 ± 3	46 ± 3	50 ± 3
Triglyceride (mg/ml)	134 ± 15	117 ± 13	139 ± 12	133 ± 21
HbA1c (%)	6.2 ± 0.3	6.0 ± 0.2	6.2 ± 0.2	6.0 ± 0.2
Systolic BP (mmHg)	129 ± 6	124 ± 5	123 ± 4	126 ± 4
CRP (mg/ml)	0.80 ± 0.26	0.11 ± 0.05*	0.74 ± 0.20	0.16 ± 0.03*
IL-8 (pg/ml)	77.9 ± 3.1†	77.6 ± 3.5	91.8 ± 5.0	76.7 ± 5.9*
BMI (kg/m^2)	22.8 ± 0.5	23.0 ± 0.6	23.5 ± 0.5	24.4 ± 0.6
Cardiac function				
LVEF (%)	54.3 ± 2.3	63.5 ± 2.7*†	53.5 ± 2.0	55.2 ± 2.6
LVEDVI (ml/m^2)	94.2 ± 3.3	93.0 ± 3.1	95.8 ± 4.9	99.1 ± 3.5
LVESVI (ml/m^2)	41.2 ± 4.4	34.0 ± 4.3	42.3 ± 4.7	43.5 ± 6.3
LVEDP (mmHg)	13.1 ± 0.9	13.8 ± 0.9	14.3 ± 1.5	14.0 ± 0.7
CI (l/min/m^2)	2.9 ± 0.1	3.0 ± 0.1	2.8 ± 0.7	2.8 ± 0.1
Medication				
ACE-I (%)	76	64	64	52
ARB (%)	24	32	36	40
Beta-blocker (%)	20	16	12	8
Calcium-channel-blocker (%)	36	44	32	40
Aspirin (%)	100	96	100	100
Ticlopidine (%)	76	20*	72	20*
SU (%)	8	8	8	12
Insulin (%)	12	12	8	8

Values represent the percent of the patients or mean ± SE. *$p < 0.05$ vs. 2 weeks; †$p < 0.05$ vs. placebo.

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BP = blood pressure; CI = cardiac index; CRP = C-reactive protein; IL = interleukin; LVEDP = left ventricular end-diastolic pressure; LVEDVI = left ventricular end-diastolic volume index; LVEF = left ventricular ejection fraction; LVESVI = left ventricular end-systolic volume index; SU = sulfonylureas; other abbreviations as in Table 1.

decreased at the acute phase of MI compared with healthy control subjects. Furthermore, by 2 weeks after MI in both the atorvastatin and placebo groups, these levels returned to levels similar to those obtained in healthy control subjects. The elevation of VEGF and PlGF levels at the acute phase of MI may be an adaptive mechanism for inducing neovascularization in ischemic myocardium (1–3,21,22) and may be caused in part through a mechanism involving hypoxia-inducible factor 1 activation (23). The decreased levels of sFlt-1, a natural antagonist of VEGF and PlGF, may be helpful for the angiogenic activity of VEGF and PlGF in ischemic myocardium in the acute phase of MI. Furthermore, the present study showed that atorvastatin treatment induced an increase in sFlt-1 levels and a reciprocal decrease in free VEGF and free PlGF levels at 6 months after MI compared with placebo treatment. Moreover, these changes in plasma levels were correlated with improvement of post-MI ventricular dysfunction from 2 weeks to 6 months after AMI. Change in LDL cholesterol levels was not related to the improvement of LV function and changes in sFlt-1, free VEGF, and free PlGF levels from 2 weeks to 6 months after AMI. Therefore, it is suggested that the increase in sFlt-1 levels and the reciprocal decrease in free VEGF and free PlGF levels after atorvastatin treatment may have clinical benefit in the chronic phase of MI in patients with AMI independently of decrease in lipid levels. However, the effect of atorvastatin-induced changes in plasma levels of sFlt-1, free VEGF, and free PlGF on long-term clinical outcome remains to be determined.

Also, the present study showed that total VEGF levels (both free and sFlt-1–bound VEGF levels) had similar changes during the study period as free VEGF levels. However, atorvastatin treatment did not affect total VEGF levels. These results indicate that atorvastatin treatment was unlikely to influence the circulating total VEGF levels which may reflect production and degradation of VEGF in the circulation. Therefore, it is possible that atorvastatin treatment may primarily increase sFlt-1 levels, resulting in a decrease in free VEGF levels, although it can not be excluded that atorvastatin could alter the binding ability of VEGF to acceptors, including sFlt-1, in the circulation.

Vascular endothelial growth factor and PlGF belong to the same gene family and play a synergistic role in pathologic angiogenesis (1–3,22). Vascular endothelial growth factor exerts its biologic activities by interacting with two receptors, Flt-1 and Flk-1/KDR, whereas PlGF interacts with Flt-1 but not Flk-1/KDR (1–4,6,7,22). It has been shown that Flk-1/KDR, expressed on vascular endothelium, mainly mediates VEGF-induced angiogenesis (1–3,22). On the other hand, Flt-1 is expressed on endothelial cells and

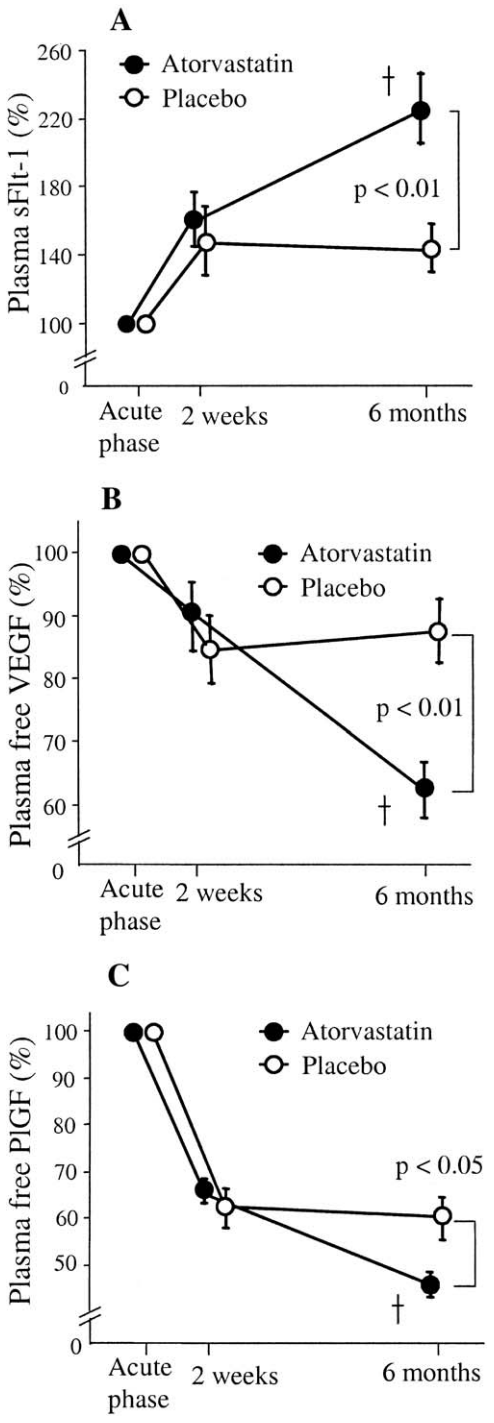

Figure 2. Comparisons of the percentage changes in the plasma levels at 2 weeks and 6 months after acute myocardial infarction relative to the levels at acute phase of myocardial infarction (=100%) between the two treatment groups. **(A)** sFlt-1; **(B)** free VEGF; **(C)** free PlGF. †$p < 0.05$ vs. respective levels at 2 weeks after MI. Abbreviations as in Figure 1.

inflammatory cells, namely, monocytes and macrophages (1–3,22). Thus, VEGF and PlGF also have proinflammatory properties, and they both thereby have the capability of promoting atherosclerosis through Flt-1. This is supported by previous reports (14,15) showing that elevated levels of VEGF and PlGF in ACS resulted in an adverse prognosis. However, it remains unclear whether elevated VEGF and PlGF levels in ACS function as a proatherogenic factor via Flt-1 or only serve as a surrogate marker of myocardial injury. Furthermore, the present study showed that percentage changes in free VEGF, free PlGF, and sFlt-1 levels were significantly correlated with the percentage changes in circulating levels of interleukin-8, a proinflammatory cytokine. It is reported (1–3,24,25) that VEGF and PlGF stimulate various inflammatory cells, including monocytes and endothelial cells, leading to production and release of interleukin-8 and other proinflammatory molecules. Also, it is known that these inflammatory cytokines locally produced in post-infarct myocardium could depress ventricular functions (26,27). Thus, the present results support previous reports (1–3,14,15) showing that VEGF and PlGF exert as proinflammatory factors, and these proinflammatory properties of VEGF and PlGF may influence post-MI ventricular function.

Soluble Flt-1, generated by alternative splicing of the Flt-1 gene, reduces effective plasma concentrations of free VEGF and free PlGF and inactivates their activities (4–11). Furthermore, sFlt-1 forms heterodimers with membrane-bound Flt-1 (4) and thus acts as a receptor blocker of Flt-1. Thus, sFlt-1 could potentially attenuate the adverse effects of VEGF and PlGF and may have a beneficial effect in ACS. In fact, a previous report (28) demonstrated that the

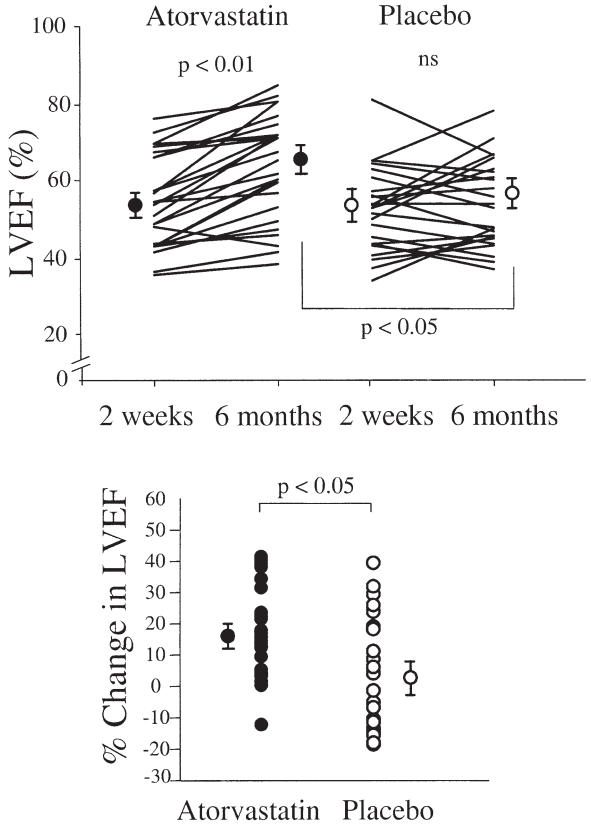

Figure 3. Comparison of changes in left ventricular ejection fraction (LVEF) from 2 weeks to 6 months after acute myocardial infarction in patients treated with atorvastatin (n = 25) and placebo (n = 25).

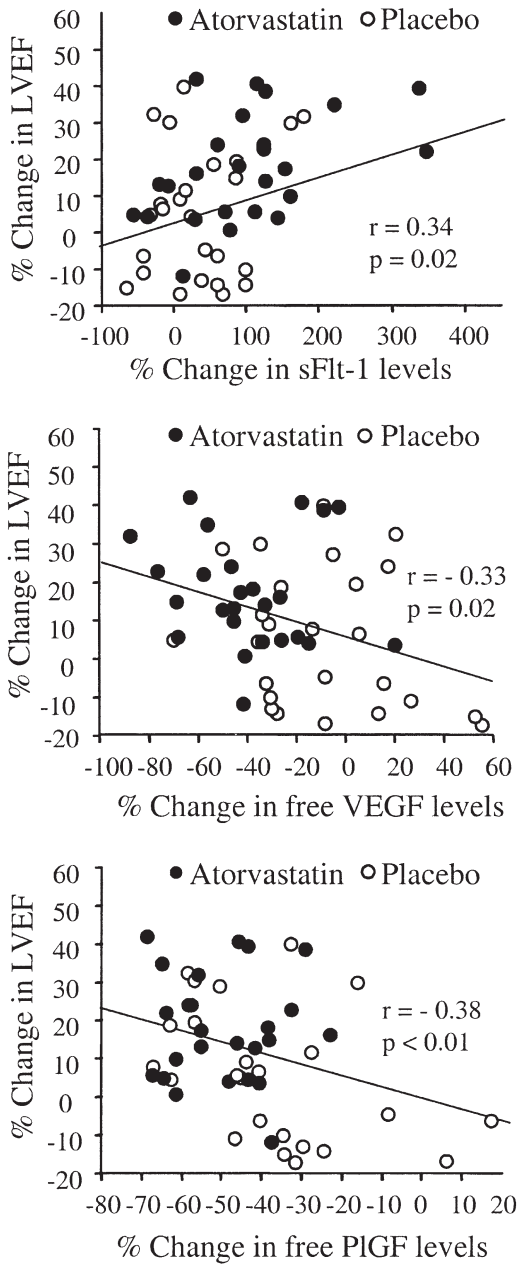

Figure 4. Correlations between percent changes in LVEF and in plasma levels of sFlt-1, free VEGF, or free PlGF from 2 weeks to 6 months after acute myocardial infarction in patients treated with atorvastatin (n = 25; **solid circles**) and placebo (n = 25; **open circles**). Abbreviations as in Figures 1 and 3.

increase in sFlt-1 plasma levels by its gene transfer inhibited vascular inflammation and prevented subsequent atherosclerosis in an animal model. On the other hand, previous reports (5,29) using an animal model showed that exogenous administration of sFlt-1 acutely induced hypertension and nephrotoxicity probably owing to inhibition of the endothelial protective action of endogenous VEGF. However, the sFlt-1 plasma levels exogenously administered were nearly 10 or 100 times higher than the physiologic plasma levels in these animal experiments (5,29). Furthermore, coronary artery disease is a long-term process in humans. Therefore, the role of elevated levels of endogenous sFlt-1 in human atherosclerotic cardiovascular disease still remains largely unknown.

Soluble Flt-1 has been shown to be expressed in vascular endothelium as well as placenta, and alternative splicing has been identified as a key regulatory step in sFlt-1 production (1–4). Although sFlt-1 expression is increased by hypoxia in placenta villous cells (11), the regulatory mechanisms by which sFlt-1 levels decreased during the acute phase of MI in the present study and thereafter increased in response to atorvastatin treatment remain unknown.

There is a marked difference in either total or free levels of VEGF levels among the different immunodetection assays (19). It is known that the measured values are considerably influenced by many factors, including characteristics of the calibrator and specificity of antibodies used in the assays (19). As shown in Figures 1B and 1D, it is expected that total VEGF levels are higher than free VEGF levels. However, the magnitude of the difference between total and free VEGF levels should be carefully interpreted because of differences in characteristics of antibodies to VEGF, calibrators, and immunoassay systems, i.e., competitive binding assay and sandwich ELISA. The PlGF levels at the chronic phase of MI in the present study were comparable with the levels in a previous report (30), but the levels were higher than those in another report (14). The reason for the difference in PlGF levels among the studies remains unclear, but it can not be excluded that racial differences or other medications in addition to statin may affect PlGF levels.

In conclusion, there was a reciprocal relationship between changes in sFlt-1 levels and free VEGF and PlGF levels after MI; atorvastatin increased sFlt-1 levels while decreasing free VEGF and PlGF levels. These changes were associated with late improvement of ventricular function after AMI, and may represent an additional benefit of statin therapy.

Reprint requests and correspondence: Dr. Kiyotaka Kugiyama, Department of Internal Medicine II, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 1110 Shimokato, Nakakoma-gun, Yamanashi, 409-3898 Japan. E-mail: kugiyama@yamanashi.ac.jp.

REFERENCES

1. Clauss M. Functions of the VEGF receptor-1 (Flt-1) in the vasculature. Trends Cardiovasc Med 1998;8:241–5.
2. Autiero M, Luttun A, Tjwa M, Carmeliet P. Placental growth factor and its receptor, vascular endothelial growth factor receptor-1: novel targets for stimulation of ischemic tissue revascularization and inhibition of angiogenic and inflammatory disorders. J Thromb Haemost 2003;1:1356–70.
3. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669–76.
4. Kendall RL, Wang G, Thomas KA. Identification of a natural soluble form of the vascular endothelial growth factor receptor, FLT-1, and its heterodimerization with KDR. Biochem Biophys Res Commun 1996; 226:324–8.

5. Maynard SE, Min JY, Merchan J, et al. Excess placental soluble Fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J Clin Invest 2003;111:649–58.
6. Kendall RL, Wang G, DiSalvo J, Thomas KA. Specificity of vascular endothelial cell growth factor receptor ligand binding domains. Biochem Biophys Res Commun 1994;201:326–30.
7. Park JE, Chen HH, Winer J, Houck KA, Ferrara N. Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR. J Biol Chem 1994;269:25646–54.
8. Levine RJ, Maynard SE, Qian C, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672–83.
9. Chung NA, Lydakis C, Belgore F, Blann AD, Lip GY. Angiogenesis in myocardial infarction. An acute or chronic process? Eur Heart J 2002;23:1604–8.
10. Blann AD, Belgore FM, Constans J, Conri C, Lip GY. Plasma vascular endothelial growth factor and its receptor Flt-1 in patients with hyperlipidemia and atherosclerosis and the effects of fluvastatin or fenofibrate. Am J Cardiol 2001;87:1160–3.
11. Hornig C, Barleon B, Ahmad S, Vuorela P, Ahmed A, Weich HA. Release and complex formation of soluble VEGFR-1 from endothelial cells and biological fluids. Lab Invest 2000;80:443–54.
12. Freedman SB, Isner JM. Therapeutic angiogenesis for coronary artery disease. Ann Intern Med 2002;136:54–71.
13. Luttun A, Tjwa M, Moons L, et al. Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by antiFlt1. Nat Med 2002;8:831–40.
14. Heeschen C, Dimmeler S, Fichtlscherer S, et al., CAPTURE Investigators. Prognostic value of placental growth factor in patients with acute chest pain. JAMA 2004;291:435–41.
15. Heeschen C, Dimmeler S, Hamm CW, Boersma E, Zeiher AM, Simoons ML, CAPTURE (c7E3 Anti-Platelet Therapy in Unstable Refractory Angina) Investigators. Prognostic significance of angiogenic growth factor serum levels in patients with acute coronary syndromes. Circulation 2003;107:524–30.
16. Bonetti PO, Lerman LO, Napoli C, Lerman A. Statin effects beyond lipid lowering—are they clinically relevant? Eur Heart J 2003;24: 225–48.
17. Ray KK, Cannon CP. Intensive statin therapy in acute coronary syndromes: clinical benefits and vascular biology. Curr Opin Lipidol 2004;15:637–43.
18. Alber HF, Dulak J, Frick M, et al. Atorvastatin decreases vascular endothelial growth factor in patients with coronary artery disease. J Am Coll Cardiol 2002;39:1951–5.
19. Jelkmann W. Pitfalls in the measurement of circulating vascular endothelial growth factor. Clin Chem 2001;47:617–23.
20. Ludwig M, Jelkmann W, Bauer O, Diedrich K. Prediction of severe ovarian hyperstimulation syndrome by free serum vascular endothelial growth factor concentration on the day of human chorionic gonadotrophin administration. Hum Reprod 1999;14:2437–41.
21. Kranz A, Rau C, Kochs M, Waltenberger J. Elevation of vascular endothelial growth factor-A serum levels following acute myocardial infarction. Evidence for its origin and functional significance. J Mol Cell Cardiol 2000;32:65–72.
22. Luttun A, Tjwa M, Carmeliet P. Placental growth factor (PlGF) and its receptor Flt-1 (VEGFR-1): novel therapeutic targets for angiogenic disorders. Ann N Y Acad Sci 2002;979:80–93.
23. Kelly BD, Hackett SF, Hirota K, et al. Cell type-specific regulation of angiogenic growth factor gene expression and induction of angiogenesis in nonischemic tissue by a constitutively active form of hypoxia-inducible factor 1. Circ Res 2003;93:1074–81.
24. Lee TH, Avraham H, Lee SH, Avraham S. Vascular endothelial growth factor modulates neutrophil transendothelial migration via up-regulation of interleukin-8 in human brain microvascular endothelial cells. J Biol Chem 2002;277:10445–51.
25. Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res 2002;53:31–47.
26. Damas JK, Gullestad L, Aass H, et al. Enhanced gene expression of chemokines and their corresponding receptors in mononuclear blood cells in chronic heart failure—modulatory effect of intravenous immunoglobulin. J Am Coll Cardiol 2001;38:187–93.
27. Nian M, Lee P, Khaper N, Liu P. Inflammatory cytokines and postmyocardial infarction remodeling. Circ Res 2004;94:1543–53.
28. Ohtani K, Egashira K, Hiasa K, et al. Blockade of vascular endothelial growth factor suppresses experimental restenosis after intraluminal injury by inhibiting recruitment of monocyte lineage cells. Circulation 2004;110:2444–52.
29. Sugimoto H, Hamano Y, Charytan D, et al. Neutralization of circulating vascular endothelial growth factor (VEGF) by antiVEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. J Biol Chem 2003;278:12605–8.
30. Werner GS, Jandt E, Krack A, et al. Growth factors in the collateral circulation of chronic total coronary occlusions: relation to duration of occlusion and collateral function. Circulation 2004;110:1940–5.